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(71) Applicant *(for all designated States except US)*: BIOTAL LTD. [GB/GB]; 5 Chiltern Close, Cardiff CF4 5DL (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MANN, Stephen, Philip [GB/GB]; 29 London Road, Harston, Cambridgeshire CB2 5QQ (GB). WARD, John, Stewart [GB/GB]; 23 Miles Court, Gwaelod-y-Garth, Mid-Glamorgan CF4 8SR (GB).

(74) Agent: GILL JENNINGS & EVERY; Broadgate House, 7 Eldon Street, London EC2M 7LH (GB).

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(54) Title: ENHANCED BIOLOGICAL DEGRADATION OF ORGANIC WASTE SYSTEMS

(57) Abstract

A method of degrading material comprising organic components, which comprises treating the material with one or more enzymes capable of degrading the organic components and with micro-organisms capable of growth on the components and on the product(s) of the enzymatic degradation and thereby generating additional enzymatic activity.

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ENHANCED BIOLOGICAL DEGRADATION OF ORGANIC WASTE SYSTEMS

Field of Invention

This invention relates to the biological degradation of agricultural, industrial, garden and domestic waste by the application of selected micro-organisms, and to enzymatically-active compositions for use in the degradation of organic wastes.

Background of the Invention

Domestic, agricultural industrial and municipal waste is a growing problem. The general increase in domestic waste, including degradable organic materials such as paper, manure, vegetable waste and grass cuttings, has led to problems of disposal. One solution is to place such organic material in large landfill sites, but this has caused a considerable problem and hazard, owing to the continuous and uncontrolled release of methane and other gases. Limited use of land available for landfill exacerbates the problem. As a result, there has been an increasing emphasis on domestic and municipal recycling, of which one route is composting of the biodegradable fraction.

An object behind the present invention is to enhance this biological degradation of the material, thereby converting it to a material more suitable for use in many aspects of horticulture, agriculture and landscaping.

The presentation of such products is undeniably important and to date it has not been proved possible to produce in a liquid form products where the enzymes and micro-organisms are present together in liquid suspensions. Historically, attempts to do this have resulted in either a rapid deterioration in the cell counts due to the incorporation of enzyme stabilisers, or the enzymes have rapidly disappeared as the bacteria have assimilated their protein matrices.

WO-A-9210945 discloses a formulation of enzymes and micro-organisms. That formulation is designed to enhance,

inter alia, the nitritive value of silage. The enzymes exclude cellulases and hemicellulases.

Summary of the Invention

According to the present invention, selected enzymes and micro-organisms are used to biologically degrade organic components. The enzymes have activities capable of degrading one or more of the components in addition to providing nutrients to the degrading bacteria. The bacterial micro-organisms are capable of growth, thereby producing additional enzyme activities, and also heat. Selected fungal components are capable of breaking down the more indigestible lignin content of woody material, providing an additional nutrient source for bacterial activity.

The invention is based on the realisation that the rate of decomposition may be increased by ensuring that sufficient numbers of selected micro-organisms are present throughout the treated system, for successful competition, and that the necessary nutrients and conditions should be present. In the process, the desirable organism will proliferate throughout the material, thereby excluding the less efficient or detrimental organisms.

The desired organisms are stabilised in the presence of the enzymes in a liquid formulation, by first fermenting the cells as a preparation of thermostable spores. The spores are stabilised in the formulation, and prevented from germination by the presence of bacteriostatic compounds and/or creation of a high osmotic pressure. The enzymes are stabilised by the presence of the same osmotic stabilisers while being protected from degradation by bacteria from the same bacteriostatic compounds.

Description of the Invention

In use of a composition of the invention, optimum conditions depend on the presence of the correct micro-organisms, and the use of the enzymes to establish rapid breakdown of, say, plant cell walls, thereby releasing nutrients for the micro-organisms. The appropriate enzymes

will break down plant and animal wastes during the composting process. During this initial breakdown, nutrients are generated to produce a medium that is conducive to the rapid proliferation of the organisms that are also provided. In addition, the growth of the bacteria achieves elevated temperatures in the degrading material, without initially affecting their numbers, but reduces undesirable components such as non-beneficial micro-organisms and weed seeds.

The enzymes will be selected according to the nature of the material to be processed. They may be adapted to the breakdown of plant cell walls and also to the breakdown of animal wastes. In predominantly woody material, the application of selected wood-degrading fungal strains will convert this normally indigestible component to a more accessible nutrient form for the bacteria.

The initial breakdown of the material having been achieved, the enzymes continue to function in increasing activities, as the temperature of fermentation rises. Enzymes and microbes having a high thermo-tolerance, e.g. above 35° or 40°C, are preferred. Enzymes capable of releasing sugars from the complete spectrum of plant and animal polysaccharides, since the components of the material will often include disaccharides and polysaccharides, are usually selected. Examples of such enzymes include cellulases, amylases, xylanases, galactanases, mannanases, arabanases, β-1,3-1,4-glucanases and the appropriate glucosidases and xylosidases. Enzymes releasing amino-acids beneficial for the biodeterioration process are also preferably included. Where a substantial proportion of the waste to be composted is of domestic origin, lipases will also usually be present in the formulation. Plant cell mass is further disrupted by the inclusion of enzymes capable of breaking down pectin and pectin esters; this has the additional advantage of allowing the micro-organisms to penetrate well into the cell biomass. In the case of leaves, a greater proportion

of the enzyme component will consist of ligninases and hemi-cellulases.

Owing to the high level of methane that is produced when they are placed in landfill sites, the composting of grass cuttings presents a particular problem. The present invention is well adapted to the composting of grass cuttings, in which case it will include enzymatic activities capable of degrading celluloses and/or hemicelluloses.

Substrates to which formulations of this invention are applied will generally contain cellulose. It is therefore particularly preferred that the novel formulation comprises cellulase, i.e. endo-1,4-β-glucanase (Enzyme Commission No. 3.2.1.4), and/or cellulose 1,4-β-cellobiosidase (EC 3.2.1.91). Xylanase activity is also particular preferred.

Other enzymes that may be present are listed in WO-A-9210945, the contents of which are incorporated herein by reference. For example, proteases and lipases may be chosen for certain substrates, while pectinase activity may be desirable for treating grass clippings.

The enzyme component can have a prolonged activity. This can be achieved by the selection of enzymes of demonstrated durability and thermostability, and of bacterial organisms that produce heat, to raise the enzymes' activity close to their thermal optimum.

Compost heap reduction and bacterial pasteurisation are also achieved in the long term by the microbial component which, as the cells proliferate, begins to produce enzymes of the types added in the original inoculum. Bacterial strains are selected for their rapid rate of growth, and their propensity to produce considerable amounts of extra-cellular enzymes. Strains may be selected for their ability to dominate a wide range of composting environments or for the ability to breakdown specific waste fractions. In woody environments, fungal strains will tend to dominate initially, before bacterial organisms contribute to the degradation process.

For the composting of materials, the inclusion of micro-organisms capable of fixing nitrogen may give an impetus to the fermentation of such materials, and improve compost quality. This may be particularly appropriate if the components and/or the enzymes are such that the enzymatic activities do not give an assimilable source of nitrogen for growth of the micro-organisms.

The micro-organisms may be facultative, anaerobic or acerobic. Aerobic organisms such as fungi may be preferred.

The micro-organisms may be selected from the genera *Bacillus, Clostridium, Streptomyces, Phaneromyces, Phanerochaete* and *Aspergillus*. Nitrogen-fixing micro-organisms may be selected from *Clostridium, Rhizobium and Azotobacter*.

Specific examples of materials which may be composted by means of the present invention are grass clippings, leaf litter, wood chippings, mixed domestic waste or municipal solid waste. Grass clippings are an example of waste which is seen as a particular problem.

For use in the invention, a composition is suitably prepared as an inoculum of the enzymes and organisms. Typical additional components of such an inoculum are nutrient additives, e.g. supplemental carbon and nitrogen sources, and inert bulking agents.

Where spore-forming organisms are a part of the formulation, such enzyme and spore preparations may be stabilised, providing that the microbial component has been prepared as near as possible to give 100% spores which are both resistant and viable. Such spores will not germinate in pure aqueous solutions, but will in the enriched solutions that are typical of liquid enzyme preparations.

Stabilisation may also be achieved by formulating products that include osmotic stabilisers such as salts or other miscible compounds, e.g. sodium chloride or glycerol, to protect the enzymes. Such additives are also able to prevent the germination of spores and thus protect the

enzyme from bacterial contamination by other invading organisms. Bateriostatic compounds (e.g. sodium benzoate) enhance the repression of bacterial spore germination, especially when the osmotic pressure required for stabilisation of enzymes is relatively low.

Similar preparations may be made and stabilised with fungi. This is achieved either by modifications to submerged fermentations, in order to induce thicker spore walls or to encourage the formation of perithicial spore structures with reduced mycelial biomass. Again, these preparations may be prevented from germination by inclusion of either or both osmotic stabilisers and other compounds to reduce germination; again, sodium benzoate may be the compound of choice.

Fungal spore preparation is often difficult, and in the case of some organisms spore formation may need to be stimulated to produce adequate numbers of spores at fermentation sizes in excess of 5 litres. For example, in the case of *Phanerochaete*, spore production is only adequately stimulated by the presence of lignin in the fermentation medium. However, spores prepared in this way may be readily stabilised in the presence of osmotic stabilisers such viscous gums (e.g. xanthan, guar, CMC etc.); each fungus has to be treated individually to maximise spore fermentation, but other factors may also have to be introduced. For example, the genus *Ophiostioma* has to undergo a period of spore wall thickening before stabilisation achieved by feeding of selected carbon sources during the final stages of fermentation.

Following these procedures, all of which may be conducted by means familiar to the skilled man, viable bacterial and fungal cells may also be preserved by freeze-drying.

The following Examples illustrate the invention.

Example 1

An inoculum was prepared from (a) enzymes capable of hydrolysing cellulose, glucans, xylans, mannans,

galactans, starch, as well as proteins and the components of pectin, and (b) two micro-organisms, one a *Bacillus* spp., the other a *Streptomyces* spp., specially selected for their enzyme and thermal productive capabilities. Sugar (sucrose) and bentonite were used to give the product bulk.

The inoculum was used to treat grass clippings, and a control experiment (without inoculum) was also run. A desirable effect was achieved by the use of inoculum, in terms of sugar production, volume reduction and temperature increase and stabilisation.

Example 2

A formulation was made comprising cellulase, 1,4-β-cellobiosidase and xylanase activities at least, thermophilic *Bacillus* sp., 1% sodium benzoate, sugar and bentonite. This formulation showed stability; a reading of c.8.0 log CFU/ml was recorded at various times over 50 weeks.

Lawn clippings from a playing field were placed in two composting containers of about 70 l capacity. One of the containers was inoculated with the formulation.

The bins were monitored for temperature, heap reduction and release of fermentable sugars as an indication of enzymatic action. It was found that the temperature in the treated bin was consistently higher over 120 hours than that of the control; the heap reduction was faster and greater after inoculation than in the control; there was an increased release of fermentable sugars in the treated bin (>26 g/l after 1 day compared with <15 g/l in the control, both decreasing to close to zero after 3 days). These results show that inoculation can positively influence the natural process of composting grass clippings.

In a second test, a mixture of garden and household waste was shredded and placed in two compost containers. One container was inoculated with the formulation.

The material was monitored for heat production and turned every 4 days until no rises in temperature were

observed. Material was followed through to completion of a dry friable humous material. The treated heap reached this mature phase in approximately 3 weeks compared to over 6 weeks for the control. Increased temperatures were recorded in the treated heap (43°C after 5 days) compared to the control (35°C after 5 days), after turning, in the first week. This shows that inoculation can positively influence the composting of mixed garden and household waste.

In a third test, farm waste consisting of used straw-based winter cattle bedding was shredded and formed into two windrows of approximately 25 tonnes each. One windrow was inoculated with a bacterial enzyme mix using a backpack sprayer as the windrow was formed.

The windrows were monitored for temperature and other physical and chemical parameters, to assess the rate at which the material was composting. Over 40 days, the treated windrow reached higher and more sustained temperatures compared to the control. This shows that inoculation with a bacterial and enzyme mix can influence the course of the natural composting of cattle bedding wastes.

In a fourth test, large windrows of approximately 30 tonnes of leaf and woody type waste were formed. One windrow was inoculated with the enzyme and bacterial mix and both windrows were monitored for temperature. The inoculated heap reached higher and more sustained temperatures (c. 65°C after 5 days, falling to c. 30°C after 23 days) than the untreated control (c. 58°C after 5 days falling to c. 20°C after 20 days).

This trial showed that the composting of wood and leaf type wastes can be influenced by the addition of a specific inoculum, and that inoculation can decrease the time required to convert this type of waste into an acceptable compost.

CLAIMS

1. A method of degrading material comprising organic components, which comprises treating the material with one or more enzymes capable of degrading the organic components and with micro-organisms capable of growth on the components and on the product(s) of the enzymatic degradation and thereby generating additional enzymatic activity.

2. A method according to claim 1, wherein the enzymes include a protease.

3. A method according to claim 1 or claim 2, wherein the enzymes include a lipase.

4. A method according to any preceding claim, wherein the enzymes include a pectinase.

5. A method according to any preceding claim, wherein the enzymes include a cellulase and/or a hemicellulase.

6. A method according to any preceding claim, wherein the material to be treated comprises horticultural or garden waste, grass clippings, leaf litter, wood chippings, mixed domestic waste or municipal solid waste.

7. A method according to claim 6, wherein the material comprises grass clippings.

8. A method according to any preceding claim, wherein the micro-organisms are selected from the genera *Azotobacter*, *Rhizobium*, *Rhizopus*, *Thermomonospora*, *Bacillus*, *Clostridium*, *Streptomyces*, *Phanerochaete*, *Ophiostoma*, *Trichoderma* and *Aspergillus*.

9. A method according to any preceding claim, wherein one or more of the micro-organisms are capable of fixing nitrogen.

10. A method according to claim 9, wherein the or each nitrogen-fixing micro-organism is selected from the genera *Bacillus*, *Clostridium*, *Rhizobium* and *Azotobacter*.

11. A method according to any of claims 8 to 10, wherein the micro-organisms include *Thermomonospora fusca* and *Phanerochaete*.

12. A method according to any preceding claim, which comprises fungal components capable of digesting lignin, cellulose and/or hemicellulose.

13. A liquid formulation comprising one or more enzymes capable of degrading organic material and one or more micro-organisms capable of growth on the product(s) of the enzymatic degradation, and in which the formulation also comprises an agent that prevents the germination of spores and, optionally, an enzyme stabiliser.

14. A formulation according to claim 13, wherein the agent prevents the proliferation of vegetable cells of micro-organisms, such as bacteria, fungi and yeasts.

15. A formulation according to claim 13 or claim 14, wherein the agent is an osmotic stabiliser and/or bacteriostat.

16. A method according to any of claims 13 to 15, wherein the agent and the enzyme stabiliser are selected from alginates, carboxymethylcellulose, xanthan gum, gum guar, sodium chloride, a benzoate, (sodium) metabisulphate, and glycerol, any of which may act to prevent spore germination and enzyme breakdown.

17. A formulation according to any of claims 13 to 16, wherein the or each micro-organism is stabilised by sporulation, before addition to the composition.

18. A formulation according to any of claims 13 to 17, which additionally comprises one, two or all of carbon sources, nitrogen sources and inert bulking agents.

19. A formulation according to any of claims 13 to 18, which additionally comprises NH_4SO_4.

20. A method according to any of claims 1 to 12, wherein the material is treated with a formulation according to any of claims 13 to 19.

INTERNATIONAL SEARCH REPORT

Int[redacted]. Application No
PCT/GB 95/00650

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C05F11/08 C05F17/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C05F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,4 032 318 (LOVNESS DONALD E) 28 June 1977 see the whole document	1-6,8-10
X A	EP,A,0 586 004 (MOLENAAR, JAN) 9 March 1994 see claims 1,5,6,8-11	1,6-10,12 2-5,12,13,18,20
A	US,A,5 145 779 (POMETTO III ANTHONY L ET AL) 8 September 1992 see claims see column 3, line 27 - line 45	1,8,11,12
A	EP,A,0 083 267 (RHONE-POULANC S.A.) 6 July 1983 see claims 1-16	1,8-10,13-16,20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 June 1995	20.06.95

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
		13-18
A	DATABASE WPI Section Ch, Week 9209 Derwent Publications Ltd., London, GB; Class A96, AN 92-068582 & JP,A,04 011 887 (CENTRAL GLASS KK) , 16 January 1992 see abstract ---	1,2, 13-16
A	DATABASE WPI Section Ch, Week 9108 Derwent Publications Ltd., London, GB; Class B04, AN 91-054447 & JP,A,03 004 791 (KANEBO KK) , 10 January 1991 see abstract -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 95/00650

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-4032318	28-06-77	CA-A-	1048290	13-02-79
EP-A-586004	09-03-94	NL-A-	9201500	16-03-94
US-A-5145779	08-09-92	NONE		
EP-A-83267	06-07-83	FR-A-	2519022	01-07-83
		AU-B-	560455	09-04-87
		AU-A-	9193582	07-07-83
		CA-A-	1179616	18-12-84
		OA-A-	7289	31-08-84
		US-A-	4755468	05-07-88